A New Method for the Rapid Analysis of Ethanol in Breath, Blood, Urine and Saliva Using the Alcolmeter Evidential Instrument

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INTRODUCTION

The introduction of legislation to control driving under the influence of alcohol has led to the development and use of a number of instrumental methods for measuring the concentration of alcohol in body fluids.11 Standard methods for blood and urine analysis, based mainly on gas chromatography, are in common use and under laboratory conditions can give results with standard deviations of better than 2 mg%.

Because of the well established equilibrium of alcohol between pulmonary blood and alveolar air, it is now common practice to measure the alcohol concentration in expired breath and to use the partition ratio to quote the blood alcohol concentration. The obvious advantages and convenience of analysing breath rather than blood have resulted in the development of a number of instruments especially for breath alcohol analysis.

Earlier instruments, such as the ‘Breathalyser’ and the ‘Photo-Electric Intoximeter’, were based on the wet chemical oxidation of the alcohol in a breath sample by a potassium dichromate/sulphuric acid mixture contained in a glass ampoule. The later development of more sensitive instrumentation, based on physical methods such as gas chromatography,12 infrared spectroscopy3 and electrochemical oxidation8-9 has made possible the better control of the discard breath volume, although in some instances the resistance to blowing still remains too high. A detailed study by Jones et al on the factors affecting the accuracy of breath measurements has shown that the ideal breath sampling system should have very little resistance to blowing and that it is desirable to discard at least 75% of the vital capacity.7 14 But, while satisfactory for laboratory use, the cost and mode of operation of some instruments based on physical methods often renders them unsuitable for routine use in the police station.

Factors such as these have been considered in the development of the ‘Alcolmeter’10 and ‘Alcosensor’4 range of instruments based on a fuel cell detector.13 Although the ‘Alcolmeter’ instruments were originally designed solely for breath analysis, the sampling system can be adapted to analyse the headspace vapours above any fluid containing alcohol, such as blood or saliva, so measuring the fluid alcohol concentration.

This communication reports on the precision and accuracy of the ‘Alcolmeter’ instruments in measuring the blood alcohol concentration by means of the analysis of a) expired breath, b) headspace vapour above blood and c) headspace vapour above saliva.

THE ‘ALCOLMETER’ INSTRUMENTS

The essential principle of the instruments, which have been previously described,8, 9 is the electrochemical oxidation of alcohol at the platinum electrode of a fuel cell. This fuel cell is an integral part of the sampling system, which is designed to aspirate a small fixed volume
of breath or headspace directly into the detector. The oxidation of alcohol in the sample generates a small voltage which is amplified and displayed in various visual forms, as illustrated by the range of instruments:

- **'Alcolmeter APST-M1'** – Alcohol preliminary screening test with small analogue meter display.
- **'Alcolmeter APST-L1'** – Alcohol preliminary screening test with coloured lights display to show various alcohol levels.
- **'Alcosensor'** – Similar to the APST-L1 and incorporating the same fuel cell sensor but manufactured in the U.S.A.4
- **'Alcolmeter AE-D1'** – Evidential instrument with digital readout. The fuel cell sensor and sampling valve are contained in a separate detachable head and heated to 60°C. This instrument may be used directly on breath, or for blood or saliva analysis by the headspace method. The result is displayed digitally and may be recorded using an optional printer.

### EXPERIMENTAL ANALYSIS

#### Liquid Blood Analysis

Liquid blood samples were analysed using a ‘Perkin Elmer F11’ gas chromatograph after dilution with a solution of n-propanol as the internal standard. The dilution (10 µl blood to 100 µl n-propanol solution) was carried out as described by Jones.6 Repeated analyses of the same blood sample showed a standard deviation of ± 0.5 mg%. The accuracy of this method, when measured using commercially available aqueous alcohol standards, was found to be better than 1.5%.

#### Breath Analysis

This was carried out using both the ‘Alcolmeter’ preliminary screening test meter instrument (APST-M1) and the ‘Alcolmeter’ evidential digital instrument (AE-D1). The subject was requested to give a steady blow through a disposable mouthpiece tube, a 1.5 cm³ portion of the end expired breath being aspirated into the fuel cell by the operator depressing the ‘read’ button on the APST-M1 or the ‘sample’ button on the AE-D1.

#### Alcohol Vapour Standards

The instruments were calibrated with a ‘Nalco’4,10 alcohol vapour standard (alcohol in argon) which had been previously standardised against the headspace taken above a known aqueous alcohol solution maintained at a constant temperature.

The precision of the evidential digital instrument was measured by noting the reading as a function of the alcohol concentration in vapour streams generated by bubbling air through aqueous alcohol synebriate standards. These standards had been prepared so as to yield vapours equal in alcohol concentration to breath in equilibrium with blood containing alcohol in the range 0–225 mg%. The standards were maintained in a water bath at 25 ± 0.1°C.

#### Headspace Blood and Saliva Analysis with the ‘Alcometer AE-D1’

Samples of blood and saliva (approx. 0.1 cm³) were collected in glass bottles (10 cm³) and each fitted with an airtight vaccine cap. A similar bottle contained an aqueous alcohol standard (approx. 0.6 cm³). All bottles were allowed to stand on the bench for 20 minutes prior
to analysis in order for the headspace vapour to equilibrate with liquid with respect to its alcohol concentration.

The AE-D1 was calibrated by attaching a hypodermic needle to the sampling port, piercing the vaccine cap of the bottle containing the standard and pressing the SAMPLE button, thereby withdrawing a fixed volume (0.8 cm³) of the headspace vapour into the fuel cell. The aqueous standard used for calibration contained 100 mg% of ethanol. For whole blood analysis the digital meter was adjusted using the CALIBRATION control to read ‘89 mg%’ and for saliva analysis to read ‘100 mg%’. Provided both the standard and the sample are at the same temperature (e.g. room temperature), then subsequent analysis of the headspace of the sample enables the weight/volume fluid alcohol concentration to be read directly off the digital meter. The value of ‘89’ for the blood calibration essentially takes into account the difference in water content between blood and saliva and is based on the ratio of the air/blood to air/water partition ratios.5

If a salt anticoagulant (e.g. fluoride/oxalate) is added to the blood to be analysed then a similar quantity should also be added to the aqueous standard used for calibration since added salt has been shown to increase the headspace alcohol concentration. Heparin is non-ionic and so does not increase the headspace alcohol concentration when used as an anticoagulant.

![Figure 1](image)

Figure 1  Relationship between ‘Alcometer AE-D1’ reading and the BAC.*

*The instrument was calibrated with the 100 mg/% standard. In vitro air alcohol/water standards were used.
Since the partition ratio of alcohol between air and water has a temperature coefficient of 6.3% °C⁻¹ it is important that both standard and sample are within 0.2°C of each other. Consequently, if many samples are to be analysed it is recommended that a thermostatted water-bath be used (± 0.1°C) because of the possible temperature fluctuation between initial calibration and analysis of the last sample.

**Blood/Breath Correlation Methods**

The subjects studied were healthy male and female laboratory staff in the 18–55 year age group. They were given alcoholic drinks of their own choosing, the doses being varied so as to obtain a wide range of blood alcohol levels. Simultaneous breath and blood samples were taken one hour after the last drink, when the majority of subjects were in the alcohol elimination phase. The instruments were all calibrated prior to commencing the tests and checked again on their completion.

The procedure adopted consisted of first taking a breath sample, immediately followed by a finger tip blood sample which was divided into aliquots for analysis by the gas chromatographic and headspace techniques. This was followed by further breath tests and the taking of a saliva sample. The time for the complete cycle of tests was less than 5 minutes so that no time correction factor for the metabolism of alcohol was necessary.

![Graph](image)

Figure 2  Regression of ‘Alcolmeter APST’ breath reading on BAC determined by gas chromatography.
RESULTS AND DISCUSSION

*Results in vitro*

The precision of the evidential ‘Alcolmeter’ was measured using aqueous alcohol synebriate standards equivalent to 5 blood alcohol concentrations in the range 0–225 mg%. The relationship of the instrument reading to the blood alcohol equivalent value is shown in Figure 1, each point being the mean of five readings. These results compare well with those obtained by Flores, in an evaluation study for the United States Department of Transportation, who reported standard deviations of 1.3, 1.3 and 2.4 mg% over 10 consecutive readings at each of 3 blood alcohol levels, 50, 100 and 150 mg% respectively.

*Results in vivo*

**Breath Analyses.** The data presented in figures 2 and 3 represent the analyses of simultaneously taken blood and breath samples for the screening test (APST-M1) and evidential (AE-D1) instruments respectively. The instruments were all calibrated with a 100 mg% ‘Nalco’ standard, assuming a blood/breath partition ratio of 2300.

The data obtained using the APST-M1 (Figure 2) on a total of 228 samples gives a linear regression equation of \( y = -2.09 + 1.031x \), as obtained by the least squares method. The standard deviation between breath and blood was 10.6 mg%, which was under the most

![Figure 3](image.png) Regression relationship between ‘Alcometer AE-D1’ breath readings and the BAC determined by gas chromatography.
favourable laboratory conditions and using co-operative subjects. This compares to a value of 12.8 mg% obtained by Alha using 10 instruments under field conditions.1

The data for the evidential instrument, shown in Figure 3, conform to the linear regression equation $y = 1.15 + 1.038x$, which demonstrates the validity of using 2300 as the blood/breath partition ratio. The standard deviation between breath/blood pairs over the whole range was 7.5 mg%.

**Headspace Blood and Saliva Analyses.** Figure 4 shows the correlation between blood analysis by the headspace technique using the AE-D1 and conventional gas chromatography for a total of 51 samples. The regression equation for this line is $y = 0.885 + 0.971x$ and the standard deviation 7.6 mg%.

Figure 5 shows the correlation between the headspace analysis of saliva and the gas chromatographic analysis of a simultaneously drawn blood sample, the total number of blood/saliva pairs being 25. The data yield a regression equation of $y = 9.26 + 0.867x$ with a standard deviation of 7.2 mg%.

The theoretical basis of measuring alcohol in the liquid phase by analysing the headspace vapour derives from Henry’s law, which states that there is a fixed ratio between the concentration of alcohol in the liquid and vapour phase at a given temperature. For in-vivo measurements this equilibrium occurs in the alveoli, with the partition ratio being close to 2.300 at an expired breath temperature of 34°C.

![Figure 4](image-url)  
*The regression relationship between analyses of blood samples by gas chromatography and the headspace technique using the ‘Alcometer AE-D1’.*
Similarly for in-vitro studies, when a volume of liquid such as blood or saliva is contained in a closed vessel the concentration of alcohol in the liquid phase can be obtained by analysis of the air above the liquid. The two conditions that must be satisfied are:
(a). The volume of the liquid must not be less than 1/100 of the volume of air space. Thus, for a 10 cm$^3$ container, the volume of blood or saliva must not be less than 0.1 cm$^3$. Provided that the volume of liquid is 0.1 cm$^3$ or more then the concentration of alcohol in the vapour phase is independent of the actual liquid volume.
(b). The standard and sample must both be at the same temperature that is within ± 0.2°C. Where many samples are to be analysed the use of a water-bath controlled to within 0.1°C is recommended.

CONCLUSION
Throughout this study the breath and the headspace readings have all been compared to the actual weight per volume analysis of alcohol in blood determined by gas chromatograph since the experimental error of this method is very low. The variability in the breath/blood data can, therefore, be largely regarded as a combination of both the breath instrument error and the variability in the breath/blood partition ratio. Similarly, for the headspace analysis

![Graph](https://example.com/graph.png)

**Figure 5** The regression relationship between analyses of blood samples by gas chromatography and simultaneously taken saliva by the headspace technique using the 'Alcometer AE-DI'.
the variability again is a combination of the instrument error and the blood/air partition ratio which, from previous *vitro* studies, has been shown to be ±4%.

Instruments such as the ‘Gas Chromatographic Intoximeter’ (G.C.I), ‘Intoxilyser’ and the ‘Alcometer AE-D1’ have probably achieved the ultimate in terms of accuracy in determining the blood alcohol concentration by breath analysis. The simplicity of the ‘Alcometer AE-D1’, coupled with the high sensitivity of its fuel cell detector, lends the instrument to field use with the added feature of a highly simplified method for blood and saliva analysis requiring no sample dilution.

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